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Improved visual discrimination learning in mice with partial 5-HT_{2B} gene deletion

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Abstract

The neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) has been linked to multiple aspects of cognition. For example, in rodents, discrimination and reversal learning are altered by experimentally induced changes in brain serotonin levels, and reduced expression of the 5-HT_{2B} receptor subtype in mice and humans is associated with decreased serotonergic tone and increased behavioral impulsivity. Serotonin modulates cognitive flexibility as well as fear and anxiety, but the specific contributions of 5-HT_{2B} receptors to these behaviors is unknown. The current study assessed mice with partial *Htr2b* deletion for performance on a touchscreen-based pairwise visual discrimination and reversal learning task followed by a test of cued fear learning. Male *Htr2b* heterozygous mice (+/−) and littermate controls (+/+) were trained to discriminate between two visual stimuli presented on a touch-sensitive screen, one which predicted delivery of a 14-mg food pellet and the other which was not rewarded. Once discrimination performance criterion was attained, the stimulus-reward contingencies were reversed. *Htr2b* +/− mice were faster to reach discrimination criterion than +/+ controls, and made fewer errors. *Htr2b* +/− mice were also slower to make responses and collect rewards. Conversely, measures of reversal learning were not different between genotypes. Pavlovian cued fear conditioning was also normal in *Htr2b* +/−mice. These data demonstrate a selective improvement in touchscreen-based discrimination learning in

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Credit Statement

AKR: Conceptualization, Investigation, Formal analysis, Writing – Original draft, Visualization; **PTP**: Formal analysis, Writing – Review and editing; **GRU**: Resources; **FSH**: Resources, Writing – Review and editing; **AH**: Conceptualization, Resources, Supervision, Writing – Review and editing

mice with partial deletion of the 5-HT_{2B} receptor, and provide further insight into the role of the 5-HT_{2B} receptor in cognition.

Keywords

serotonin; behavioral flexibility; touchscreen; fear; discrimination; learning

Introduction

The neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) has been suggested to mediate cognitive processes that contribute to flexible behavior [1,2]. Across species, behavioral flexibility is commonly assessed using assays of discrimination and reversal learning, where animals first learn to discriminate between distinct stimuli, and then must reverse this learned contingency. Serotonin depletion has been shown to facilitate the acquisition of a conditional visual discrimination in rodents [3,4], an effect that appears to be mediated by faster correct responding and reduced proactive interference from preceding trials [4,5].

In line with these observations, increasing serotonin levels with monoamine oxidase inhibitors impairs learning of a spatial discrimination [6,7]. Further, manipulations that increase serotonergic tone, such as genetic deletion of the serotonin transporter (SERT) and chronic fluoxetine treatment, reduce errors made during reversal learning [8]. Reversal learning is also impaired by depletion of cortical, but not striatal, serotonin as rodents fail to inhibit responses toward a previously rewarded stimulus [9–11]. Thus, these findings suggest that serotonin slows discrimination learning while facilitating flexibility during reversal. These effects may be mediated by multiple processes, including serotonergic control of response inhibition, reward sensitivity, negative feedback sensitivity, or general outcome tracking [2].

Serotonin acts on a diverse class of G-protein coupled and ligand-gated receptors. Although many of these receptors have been shown to regulate behavioral flexibility [12–16], relatively little is known regarding the contribution of 5-HT_{2B} receptors to this process. 5-HT_{2B} receptors are G-protein coupled receptors and are found in several brain regions involved in cognitive functions, including the raphe nuclei, hypothalamus, amygdala, hippocampus, septum, and cerebellum [17–19]. Interestingly, somatodendritic stimulation of G-q coupled 5-HT_{2B} receptors in the dorsal raphe nuclei increases serotonergic tone [19–21], suggesting a variety of mechanisms through which this receptor subtype could modulate behavioral flexibility.

Prior studies have found that *HTR2B* knockout, produced by a heterozygous stop codon in this gene, is associated with higher rates of human impulsive behavior as well as psychosis and early-onset schizophrenia [22,23]. Various other *HTR2B* polymorphisms have been associated with a constellation of neurobehavioral dysfunctions, including drug abuse, aggression, and impulsive behavioral profiles [24–26]. In mice, constitutive deletion of the *Htr2b* gene leads to a number of behavioral alterations similar to those associated with the human *HTR2B* variants, including increased impulsivity, hyper-responsivity to novelty, deficient sensorimotor gating, social impairment and abnormal performance on various

measures of learning, including fear learning [22,27]. Whether Htr2b contributes to flexible reward learning has not yet been investigated. Here, we sought to extend the existing literature on serotonin's role in behavioral flexibility and fear learning by assessing the performance of mice heterozygous for a *Htr2b* null mutation on a touchscreen-based pairwise visual discrimination and reversal learning task and a test of cued fear learning.

Materials and methods

Subjects

Male *Htr2b* heterozygous (+/−) mice (n = 7) and wild-type littermate controls (*Htr2b* +/+, n = 9) were bred from founder mice (+/+ bred with +/−) received from the Mutant Mouse Resource & Research Centers Depository (MGI: 3604521). This line was developed by Deltagen using 129P2/OlaHsd ES cells that were backcrossed to produce a congenic C57BL/6J knockout mouse line. We have previously demonstrated robust performance on touchscreen-based tasks in the C57BL/6J strain [28,29]. All mice were bred at the National Institute on Drug Abuse Intramural Research Program (Baltimore, Maryland) and transferred to the National Institute on Alcoholism and Alcohol Abuse Intramural Research Program (Rockville, MD) for testing. Mice averaged 190 days of age (range 140–260 days) at the time of testing and genotypes were matched for age and free-feeding weight at the start of testing. Mice were housed in groups of 2–4 for the duration of the experiment on a 12-h light-dark cycle and tested during the light phase of the cycle. All experimental procedures were performed in accordance with the Guide for Care and Use of Laboratory Animals [30] and approved by the Animal Care and Use Committee of the National Institute on Alcoholism and Alcohol Abuse Intramural Research Program.

Discrimination and reversal testing

Apparatus and pre-training.—Prior to training, all mice were food restricted (85% of free-feeding weight) to motivate responding for the food reward. Mice underwent visual discrimination and reversal learning training using the Bussey-Saksida Touch Screen System (model 80614, Lafayette Instruments, Lafayette, IN, USA), as previously described [28,31]. A 14-mg food pellet served as the reward (#F05684, BioServ, Frenchtown, NJ, USA).

Discrimination.—Two novel 6.5 cm^2 stimuli ('fan' and 'marbles') were presented simultaneously in a spatially randomized manner. For all mice, responses at the 'fan' stimulus were reinforced continuously (100% probability of reward) while responses at the 'marble' stimulus (='errors') produced no food reward and a 15-s 'timeout' period during which the house light was turned off and reinforcers could not be earned. The designation of the correct stimulus was not counterbalanced across mice as we have found the fan and marble stimuli to be preferred equally by C57BL/6J male mice (e.g., Radke et al., 2019). Each error was followed by a correction trial ("correction") in which the two stimuli were presented in the same spatial configuration. The next trial proper could not begin until a correct response was made on a correction trial. Mice were given the opportunity to complete 30 trials (excluding correction trials) per daily 60-min session until they reached a performance criterion of ≥85% correct responses on two consecutive sessions.

Reversal.—Here, the designation of stimuli as correct versus incorrect was reversed for each mouse such that the correct stimulus was ‘marble’ and the incorrect stimulus was ‘fan.’ Mice underwent daily reversal training sessions (30 trials/day) until a criterion of ≥85% correct responding (excluding correction trials) over two consecutive sessions was met.

Cued fear conditioning

Four weeks after the completion of reversal learning, mice underwent fear conditioning and testing as described previously [32,33]. Following 180 s of acclimation to the chamber, mice received three pairings of the conditioned stimulus (CS) (30-s, 80-dB white noise cue) and unconditioned stimulus (US) (a co-terminating, 2-s, 0.6-mA scrambled footshock) with an inter trial interval (ITI) of 60 to 90 s. 24-h later fear learning was tested in a new context. Following a 180-s acclimation period, the CS was presented four times for 30 s (5-s ITI).

Statistical analysis

For discrimination and reversal learning, dependent measures included sessions to criterion, total trials, percent correct responses (=100*(correct choices/total choice)), total errors, total correction trials (=corrections), and latency to choice (correct and incorrect), and latency to collect reward. To examine choice strategy, individual trials during each discrimination and reversal session were categorized as wins (=correct choice) or losses (=incorrect choice) [28]. Choice on trials following each win or loss were then classified either as a win-stay (correct following correct), or a lose-shift (correct following incorrect). The percentage of win-stay trials was calculated as follows: (# of win-stay trials / total # of wins) * 100. The percentage of lose-shift trials was calculated as follows: (# of lose-shift trials / total # of losses) * 100. Data from discrimination and reversal learning were analyzed by comparing genotypes with t-tests corrected for multiple comparisons with the Holm-Sidak method and adjusted p-values are reported. Sessions to criterion was analyzed by comparing survival curves with a Log-rank test.

In the fear conditioning task, % freezing was calculated (=[amount of time freezing/total time of baseline or CS] × 100). Due to a technical problem, data from three mice (two +/+ and one +/−) were lost from the fear conditioning session. Data were analyzed with RM two-way ANOVA with genotype and trial as factors and *post hoc* Dunnett’s test. All data were analyzed in GraphPad Prism (v8) with an alpha level of 0.05. Data are expressed as means ± standard error of the mean in all figures and tables.

Results

Htr2b +/− mice showed facilitated discrimination learning

Htr2b +/− mice reached discrimination criterion in fewer sessions than +/+ controls, as evidenced by a difference in the session-to-criterion survival curves in the genotypes (Log-rank test, $\chi^2(1, n = 16) = 5.683$, $P = 0.017$) (Figure 1A) and the number of sessions ($t_{14} = 2.502$, $P = 0.050$). *Htr2b* +/+ mice averaged 12.56 ± 1.08 sessions on discrimination and +/− mice averaged 8.43 ± 1.25. The total number of trials ($t_{14} = 3.021$, $P = 0.036$) and errors ($t_{14} = 2.759$, $P = 0.045$) completed were also lower in *Htr2b* +/− mice relative to +/+ controls (Figure 1B). Correction trials did not differ between the genotypes ($t_{14} = 1.671$, $P = 0.117$)

(Figure 1B). The average number of trials, errors, and corrections completed per session were not different between genotypes (P > 0.05 for all). *Htr2b* +/− mice were slower to collect rewards (t_{14} = 2.547, P = 0.023) and make correct (t_{14} = 4.928, P < 0.001) and incorrect (t_{14} = 5.457, P < 0.001) responses (Figure 1C). Lastly, the percent of win-stay and lose-shift trials completed during discrimination were not affected by genotype (Figure 1D).

Htr2b +/− mice show normal reversal learning

There were no genotype differences in session-to-criterion survival curves (Log-rank test, $\chi^2(1, n = 16) = 0.012$, P = 0.913) (Figure 2A) or sessions to reach reversal criterion (t_{14} = 0.382, P = 0.961). *Htr2b* +/+ mice averaged 15.22 ± 3.95 sessions on reversal and +/− mice averaged 13.43 ± 1.45. Total trials (t_{14} = 0.293, P = 0.961), errors (t_{14} = 0.447, P = 0.961) and correction trials (t_{14} = 0.679, P = 0.942) were also not different between the genotypes (Figure 2B). The average latency to make an incorrect response was higher in *Htr2b* +/− mice than +/+ controls (t_{14} = 2.740, P = 0.047) but though also trending higher, the latency to make a correct response (t_{14} = 2.213, P = 0.086) and collect the reward (t_{14} = 1.858, P = 0.086) was not significantly higher in the *Htr2b* +/− mice (Figure 2C). The percent of win-stay and lose-shift trials completed during reversal did not differ by genotype (Figure 2D).

Htr2b +/− mice show normal conditioned fear learning

Mice acquired fear learning (main effect of trial: $F_{3,36}$ = 17.82, P < 0.001) but there were no differences between *Htr2b* +/− and control mice (main effect of genotype: $F_{1,12}$ = 0.255, P = 0.623; trial x genotype interaction: $F_{3,36}$ = 0.424, P = 0.737) (Figure 3A). When data were collapsed across genotype, freezing during the second (P < 0.001) and third (P < 0.001) trials were significantly higher than at baseline (*post-hoc* Dunnett's test).

Mice of the different genotypes froze at similar levels during a test of fear learning (main effect of genotype: $F_{1,15}$ = 0.25, P = 0.823; trial x genotype interaction: $F_{4,60}$ = 0.530, P = 0.714) (Figure 3B). There was a significant main effect of trial ($F_{4,60}$ = 5.924, P < 0.001). When data were collapsed across genotype, *post-hoc* comparisons (Dunnett's test) revealed that freezing was significantly higher than baseline during the first (P < 0.001), second (P = 0.001), and third (P = 0.009) trials (trial 4: P = 0.072).

Discussion

The main finding of the current study was that male *Htr2b* +/− mice exhibit improved learning on a visual discrimination task. *Htr2b* +/− mice required fewer sessions, trials, and errors to reach the discrimination criterion, as compared to *Htr2b* +/+ control mice. This phenotype was specific to discrimination, as subsequent reversal learning was normal in *Htr2b* +/−mice. Pavlovian fear conditioning was also normal in these mice.

The mechanisms underlying the facilitation of discrimination learning in *Htr2b* +/− mice are unclear. One possible explanation for the enhanced visual discrimination observed here in *Htr2b* +/− mice is that partial loss of the 5-HT_{2B} receptor may indirectly decrease serotonergic tone [20], which has been previously shown to improve discrimination learning [3,4]. A recent *ex vivo* electrophysiology study found that application of a 5-HT_{2B} receptor agonist increased serotonin neuronal firing in the dorsal raphe nucleus (DRN). Based on this

and other observations, the authors suggest that somatodendritic serotonin release in the DRN stimulates Gq-coupled 5-HT_{2B} receptors resulting in a positive feedback loop that increases serotonergic tone [20]. This mechanism is thought to contribute to the role of 5-HT_{2B} receptors in the antidepressant actions of selective serotonin reuptake inhibitors [21] and other drugs targeting SERT [34]. If 5-HT_{2B} activation potentiates serotonergic signaling, then lesser *Htr2b* gene expression in *Htr2b* +/− mice could have decreased basal extracellular serotonin levels and reduced serotonin release events. Because the current study is, to our knowledge, the first examination of heterozygous *Htr2b* null mutant mice, this mechanism remains speculative as an explanation of the improved discrimination learning in these mice. It is, however, supported by earlier work showing that reductions in serotonin signaling facilitate this form of learning [3–5]. Further work is needed to determine the precise nature of serotoninergic abnormalities in *Htr2b* +/− mice.

Serotonin levels, particularly in the orbitofrontal and prefrontal cortices, positively correlate with reversal learning performance [35–37] and experimentally increasing serotonin signaling promotes reversal learning [2,8,10,11]. Given that partial deletion of the 5-HT_{2B} receptor may indirectly decrease serotonin release, these prior findings predict reversal learning might be impaired in *Htr2b* +/− mice. However, these mice did not display abnormalities in reversal performance in the touchscreen-based task. These negative data suggest that, although 5-HT_{2B} receptors may be involved in initial rule learning, they may be less critical for intra-dimensional shifting within the same rule, possibly because partial *Htr2b* deletion does not reduce serotonin levels sufficiently to alter reversal learning. In this context, the current data show that *Htr2b* +/− mice exhibit normal conditioned fear, whereas a previous study found fear conditioning deficits in full *Htr2b* −/− mice [27]. It will therefore be interesting to test whether *Htr2b* −/− mice display deficits in reversal learning.

We have previously shown that dopamine and striatal regions in which dopamine signals contribute to performance in the touchscreen-based discrimination [28,38,39] and reversal [29] tasks employed in the current study. For example, we found impaired discrimination performance and slower response latencies in mutant mice with reduced phasic dopamine neuron firing due to deletion of the NMDA NR1 subunit on dopaminergic neurons [39]. These findings are pertinent to the current study in that the 5-HT_{2B} receptor has been shown to alter dopaminergic signaling and other neurotransmitter systems relevant to behavioral flexibility [40,41]. 5-HT_{2B} receptors regulate dopamine release in the nucleus accumbens [40,41] and targeted deletion of the receptor on mesolimbic dopamine neurons alters the firing of those cells [42]. Tissue levels of dopamine and dopamine type-2 receptor mRNA expression are decreased in the dorsal striatum of *Htr2b* −/−mice [27]. If present, similar alterations in dopaminergic signaling in *Htr2b* +/− mice could account for the slower response and reward retrieval latencies observed here. Of interest, this latter finding was unexpected as baseline locomotion is preserved in mice with full *Htr2b* deletion while the locomotor response to novelty and psychostimulants in enhanced [27,34]. Further studies will be needed to determine whether longer latencies represent motivational or attentional deficits.

It is important to recognize that the results reported here may be influenced by factors such as the age or sex of the mice. Due in part to the extended nature of the discrimination and

reversal learning tasks, some mice were approaching 10 months of age by the end of this study. Further, these experiments were only conducted in male mice. It is, unfortunately, difficult to speculate whether these results would also translate to female mice, as there is very little data on reversal learning in females. It should also be noted that the sample size used here might also not have been large enough to detect some effects, such as the trend for increased correct response latencies during reversal.

In summary, partial gene deletion of the serotonin *Htr2b* receptor subtype in male mice results in improved touchscreen discrimination learning, without concomitant alterations in reversal learning or Pavlovian fear conditioning. These findings provide further evidence for a role for this receptor subtype in cognition, with implications for understanding how 5-HT_{2B} receptor abnormalities might contribute to the pathophysiology of disordered cognition in psychiatric disease.

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Highlights

- 5-HT_{2B} receptors are implicated in cognitive function and regulation of serotonin signaling
- Male mice with partial *Htr2b* deletion were tested for discrimination, reversal, and fear learing
- *Htr2b* +/− mice were faster to reach discrimination criterion than +/+ controls and made fewer errors
- Reversal and fear learning were normal in *Htr2b* +/−mice

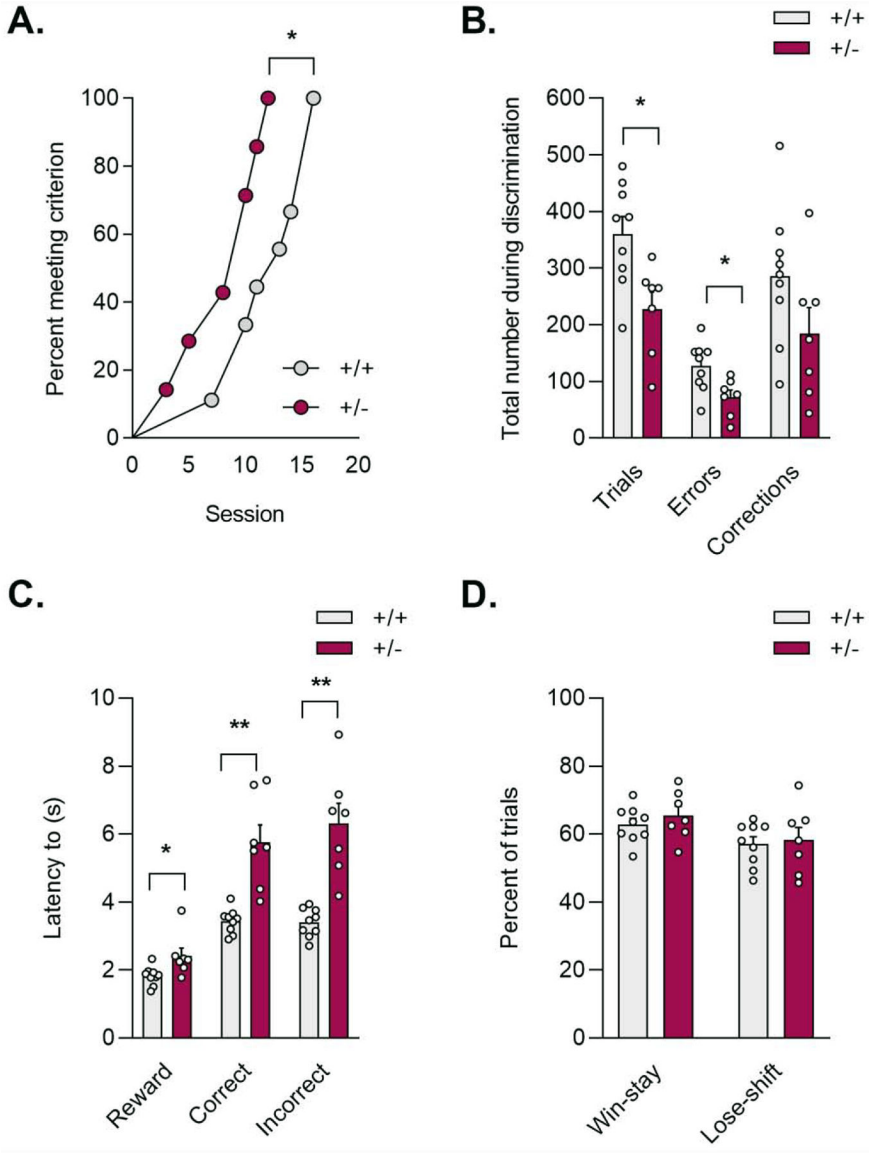

Figure 1: Htr2b +/− mice showed facilitated discrimination learning.

(**A**) Survival analysis of sessions to discrimination criterion suggests improved performance in *Htr2b* +/− mice. (**B**) Total trials, errors, and correction trials completed during all discrimination sessions in control +/+ mice (light gray) and +/− (red) mice. (**C**) Latency to collect rewards and make correct and incorrect responses during all discrimination sessions. (**D**) Percent of win-stay and lose-shift trials for discrimination. Data are expressed as mean ± SEM. **P* < 0.05, **P < 0.01 versus *Htr2b* +/+ controls.

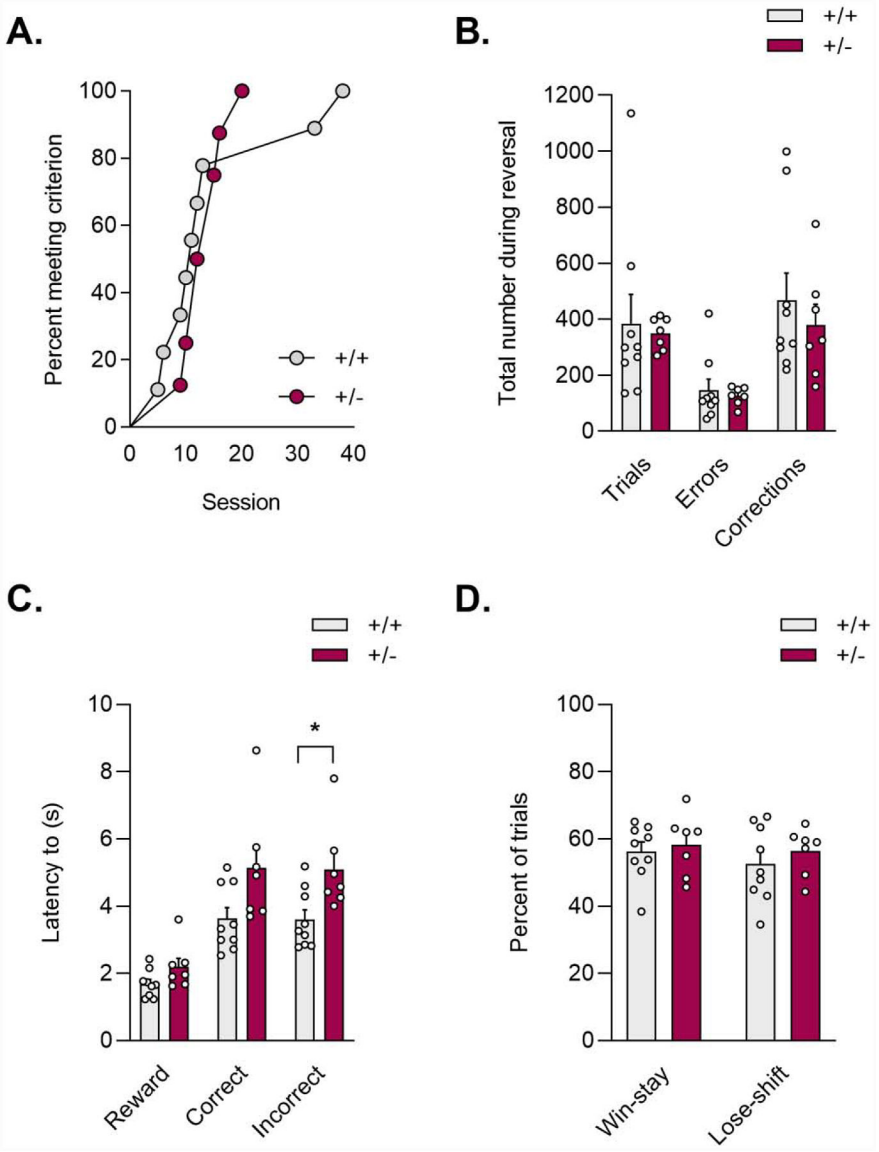

Figure 2: Htr2b +/− mice show normal reversal learning.

(**A**) Survival analysis of sessions to reversal criterion suggests *Htr2b* +/− mice perform similar to controls. (**B**) Total trials, errors, and correction trials completed during all reversal sessions in control +/+ mice (light gray) and +/−(red) mice. (**C**) Latency to collect rewards and make correct and incorrect responses during all reversal sessions. (**D**) Percent of win-stay and lose-shift trials for reversal. Data are expressed as mean ± SEM. **P* < 0.05, versus *Htr2b* +/+ controls.

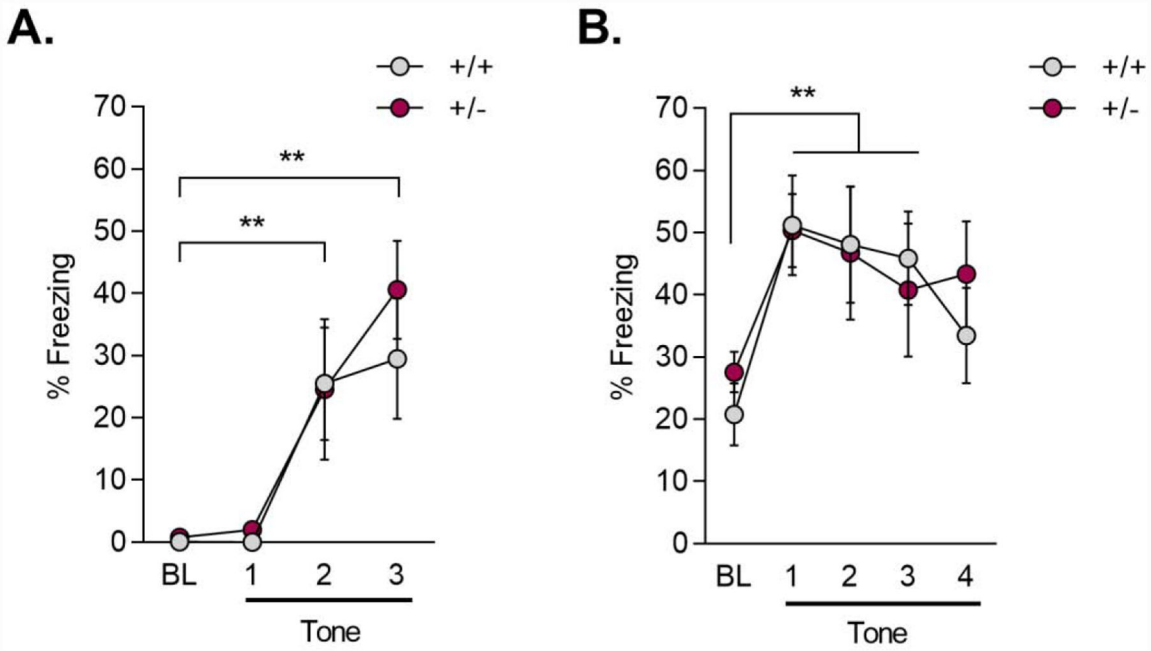

Figure 3: Htr2b +/−mice show normal conditioned fear learning.

(**A**) Acquisition of fear was similar between genotypes, as evidence by freezing following three CS presentations. (**B**) Freezing was also similar between genotypes during a test of fear learning. Data expressed as mean ± SEM. *$P < 0.05$, **$P < 0.01$ versus baseline (BL).